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Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective

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INTRODUCTION

In 1946, Claude E. ZoBell reviewed the action of microorganisms on hydrocarbons (298). He recognized that many microorganisms have the ability to utilize hydrocarbons as sole sources of energy and carbon and that such microorganisms are widely distributed in nature. He further recognized that the microbial utilization of hydrocarbons was highly dependent on the chemical nature of the compounds within the petroleum mixture and on environmental determinants.

Twenty-one years after ZoBell's classic review, the supertanker Torrey Canyon sank in the English Channel. With this incident, the attention of the scientific community was dramatically focused on the problems of oil pollution. After this event, several studies were initiated on the fate of petroleum in various ecosystems. The expansion of petroleum development into new frontiers, such as deep offshore waters and ice-dominated Arctic environments, and the apparently inevitable spillages which occur during routine operations and as a consequence of acute accidents have maintained a high research interest in this field.

The chemically and biologically induced changes in the composition of a polluting petroleum hydrocarbon mixture are known collectively as weathering. Microbial degradation plays a major role in the weathering process. Biodegradation of petroleum in natural ecosystems is complex. The evolution of the hydrocarbon mixture depends on the nature of the oil, on the nature of the microbial community, and on a variety of environmental factors which influence microbial activities.

Attention has been focused on marine environments since the world's oceans are the largest and ultimate receptors of hydrocarbon pollutants. Most previous reviews concerning the microbiology of petroleum pollutants have been concerned with the marine environment (6, 15, 24, 34, 76, 87, 107-110, 161a, 165, 209, 267, 298, 300, 301, 304). This review expands the scope to include consideration of the fate of petroleum hydrocarbons in freshwater and soil ecosystems. It also discusses several case histories relevant to the role of microbial degradation in determining the fate of petroleum pollutants from major oil spills.

CHEMISTRY OF PETROLEUM BIODEGRADATION

Degradation of Individual Hydrocarbons

Petroleum is an extremely complex mixture of hydrocarbons. From the hundreds of individual components, several classes, based on related structures, can be recognized. The petroleum mixture can be fractionated by silica gel chromatography into a saturate or aliphatic fraction, an aromatic fraction, and an asphaltic or polar fraction (48). Several studies have been performed to determine the metabolic pathways for degradation of these compounds, and there have been a number of reviews on this subject (103,

112, 113, 122, 123, 144, 190, 198, 209, 216, 217, 222, 237, 259, 266, 268).

Hydrocarbons within the saturate fraction include *n*-alkanes, branched alkanes, and cycloalkanes (naphthenes). The *n*-alkanes are generally considered the most readily degraded components in a petroleum mixture (91, 169, 197, 265, 298). Biodegradation of *n*-alkanes with molecular weights up to *n*-C_{44} have been demonstrated (137). The biodegradation of *n*-alkanes normally proceeds by a monoterminal attack; usually a primary alcohol is formed followed by an aldehyde and a monocarboxylic acid (112, 113, 198, 201, 228, 268, 270, 299). Further degradation of the carboxylic acid proceeds by β-oxidation with the subsequent formation of two-carbon-unit shorter fatty acids and acetyl coenzyme A, with eventual liberation of CO_2. Fatty acids, some of which are toxic, have been found to accumulate during hydrocarbon biodegradation (16, 172). Omega (diterminal) oxidation also has been reported (162). Subterminal oxidation sometimes occurs, with formation of a secondary alcohol and subsequent ketone, but this does not appear to be the primary metabolic pathway utilized by most *n*-alkane-utilizing microorganisms (190). A new pathway recently was elucidated by W. R. Finnerty (personal communication), who found that an *Acinetobacter* species can split a hydrocarbon at the number 10 position, forming hydroxy acids. The initial steps appear to involve terminal attack to form a carboxylic acid, subterminal dehydrogenation at the number 10 position to form an unsaturated acid, and splitting of the carbon chain to form a hydroxy acid and an alcohol.

Highly branched isoprenoid alkanes, such as pristane, have been found to undergo omega oxidation, with formation of dicarboxylic acids as the major degradative pathway (199, 222, 223). Methyl branching generally increases the resistance of hydrocarbons to microbial attack (105, 197, 222, 241). Schaeffer et al. (241), for example, found that terminal branching inhibits biodegradation of hydrocarbons. Methyl branching at the beta position (*anteiso*-terminus) blocks β-oxidation, requiring an additional strategy, such as alpha oxidation (35, 188), omega oxidation (222), or beta alkyl group removal (57, 248).

Cycloalkanes are particularly resistant to microbial attack (103, 213, 217, 259, 266). Complex alicyclic compounds, such as hopanes (tripentacyclic compounds), are among the most persistent components of petroleum spillages in the environment (18). There have been several reports of the direct oxidative and co-oxidative degradation of both substituted and unsubstituted cycloalkanes. The microbial metabolism of cyclic hydrocarbons and related compounds has been reviewed by Perry (27). Up to six-membered condensed ring structures have been reported to be subject to microbial degradation (73, 281). Several unsubstituted cycloalkanes, including condensed cycloalkanes, have been reported to be substrates for co-oxidation with formation of a ketone or alcohol (35–37, 217). Once oxygenated, degradation can proceed with ring cleavage. Degradation of substituted cycloalkanes appears to occur more readily than the degradation of the unsubstituted forms, particularly if there is an *n*-alkane substituent of adequate chain length (217, 256). In such cases, microbial attack normally occurs first on the substituted portion, leading to an intermediate product of cyclohexane carboxylic acid or a related compound. A novel pathway for the degradation of cylohexane carboxylic acid involves formation of an aromatic intermediate (217) followed by cleavage of the aromatic ring structure.

The degradation of aromatic hydrocarbons has been reviewed by Gibson and others (85, 122, 123, 125–127, 144, 237). The bacterial degradation of aromatic compounds normally involves the formation of a diol followed by cleavage and formation of a diacid such as *cis,cis*-muconic acid. In contrast, oxidation of aromatic hydrocarbons in eucaryotic organisms has been found to form a *trans*-diol. For example, fungi have been shown to oxidize naphthalene to form *trans*-1,2-dihy xy-1, 2-dihydronaphthalene (59, 60, 66, 106). The results indicate that only one atom of molecular oxygen is incorporated into the aromatic nucleus, as has been found for mammalian aryl hydrocarbon hydroxylase systems. Cerniglia and Gibson (62, 63) and Cerniglia et al. (65, 66) have investigated the fungal oxidation of polynuclear aromatic hydrocarbons. They found evidence for formation of *trans*-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene by *Cunninghamella elegans* from the oxidation of benzo(a)pyrene. Cerniglia and Gibson (61) and Cerniglia et al. (64, 68, 69) also investigated the metabolism of naphthalene by cyanobacteria. They found that naphthalene was oxidized in the light but not in the dark. *Scenedesmus* strains also were shown to utilize *n*-heptadecane in the light (mixotrophic growth), but were unable to utilize this alkane in the dark (191). The major product formed by *Agmenellum* and *Oscillatoria* species was 1-naphthol (61). These organisms also formed *cis*-1,2-dihydroxy-1,2-dihydronaphthalene and 4-hydroxy-1-tetralene (68). These results suggest that cyanobacteria have a variety of mechanisms for initiating the oxidation of naphthalene. The *Oscillatoria* species also has been found to oxidize biphenyl, indicating that a wider range of aromatic hydro-

carbons are subject to oxidation by cyanobacteria (69).

Light aromatic hydrocarbons are subject to evaporation and to microbial degradation in a dissolved state (163, 164). Extensive methyl substitution can inhibit initial oxidation (18, 85). Initial enzymatic attack may be on the alkyl substituent or, alternatively, directly on the ring (123). Condensed ring aromatic structures are subject to microbial degradation by a similar metabolic pathway as monocyclic structures (85, 96, 124, 302); condensed ring aromatic hydrocarbons, however, are relatively resistant to enzymatic attack; for example, Lee and Ryan (180) found that biodegradation rates were over 1,000 times higher for naphthalene than for benzopyrenes. Structures with four or more condensed rings have been shown to be attacked, in some cases, by co-oxidation or as a result of commensalism (30, 85, 124, 275, 284, 286).

The metabolic pathways for the degradation of asphaltic components of petroleum are probably least well understood. These are complex structures which are difficult to analyze with current chemical methodology. The degradation of various sulfur-containing components of petroleum has been examined (151, 176, 208, 286, 296), but no uniform degradative pathway, comparable to the pathways established for aliphatic and aromatic hydrocarbons, has yet emerged for the asphaltic petroleum components. Advances in determining degradative pathways for asphaltic petroleum components are dependent on improved chemical analytical methodology. The elucidation of the biochemical fate of asphaltic petroleum compounds is a major challenge for future research on petroleum biodegradation.

Another important future research need involves determining the importance in the environment of the various pathways for hydrocarbon biodegradation. It is clear that various biochemical strategies exist for the microbial utilization of petroleum hydrocarbons. What remains to be done is to detect intermediate products in natural environments that receive petroleum hydrocarbons to determine which pathways are actively used by microbial populations in natural ecosystems. It is likely, but as yet unproven, that different pathways will be active under different conditions, e.g., at different hydrocarbon concentrations.

Degradation of Hydrocarbons Within Petroleum Mixtures

The qualitative hydrocarbon content of the petroleum mixture influences the degradability of individual hydrocarbon components. Walker et al. (287) examined the susceptibility to microbial degradation of hydrocarbons in weathered crude and fuel oils. They reported far less degradation in a heavy no. 6 fuel oil (Bunker C oil) than in a light no. 2 fuel oil (heating oil and diesel fuel) and less degradation in a heavy Kuwait crude oil than in a light south Louisiana crude oil. They reported major differences in the susceptibility to degradation of each of the components (identical compounds) within the context of the different hydrocarbon mixtures of the oils tested.

Mulkins-Phillips and Stewart (206) found that *n*-alkanes within a Venezuelan crude oil were degraded less than the same *n*-alkanes within an Arabian crude oil. Westlake et al. (292) examined the effect of crude oil composition on petroleum biodegradation. The ability of mixed microbial populations to utilize the hydrocarbons in four crude oils as the sole carbon source was found to depend not only on the composition of the unsaturated fraction but also on that of the asphaltic fraction. Using an oil which lacked a normal *n*-alkane component, they demonstrated that the aromatic fraction of oil was capable of sustaining bacterial growth. Horowitz et al. (148) used the technique of sequential enrichment to isolate organisms which could utilize progressively more complex (i.e., resistant to microbial degradation) compounds.

Several investigators have examined the potential activities of hydrocarbon-degrading bacteria by using ^{14}C-radiolabeled hydrocarbons. Caparello and LaRock (58) described an enrichment method for quantifying the activity of hydrocarbon-oxidizing bacteria in water and sediment that used [^{14}C]hexadecane. They found that the hydrocarbon-oxidizing potential of environmental samples reflects the hydrocarbon burden of the area and the ability of the indigenous microorganisms to utilize hydrocarbons. Walker and Colwell (279) observed that rates of mineralization were greater for hexadecane than for naphthalene, which were greater than those for toluene, which were greater than those for cyclohexane. Greater rates of uptake and mineralization were observed for bacteria and samples collected from an oil-polluted harbor than for samples from a relatively unpolluted region. They reported turnover times of 15 and 60 min for the polluted and unpolluted areas, respectively, using [^{14}C]hexadecane. Roubal and Atlas (239) found that biodegradation potentials follow the order hexadecane > naphthalene $\gg$ pristane > benzanthracene. Lee (179) found that alkanes and low-molecular-weight aromatics (benzene, toluene, naphthalene, and methylnaphthalene) were degraded to CO_2 by microorganisms in river water, but that higher-molecular-weight aromatics were relatively resistant

to microbial degradation. Herbes and Schwall (140) found that polyaromatic hydrocarbon turnover times in petroleum-contaminated sediments increased from 7.1 h for naphthalene to 400 h for anthracene, 10,000 h for benz(a)anthracene, and more than 30,000 h for benz(a)pyrene. Polynuclear aromatic compounds tended to be only partially, rather than completely, degraded to CO_2.

Two processes which need be considered in the metabolism of petroleum hydrocarbons are co-oxidation and sparing. Both processes can occur within the context of a petroleum spillage. LePetit and Tagger (186), for example, found that acetate, an intermediate product in hydrocarbon biodegradation, reduced the utilization of hexadecane. A diauxic phenomenon has been reported for the degradation of pristane, in which pristane was not degraded in the presence of hexadecane (223). The basis for this sparing effect was not defined, and it is not known whether this is an example of classical catabolite repression. Similar sparing effects undoubtedly occur for other hydrocarbons. Such diauxic phenomena do not alter the metabolic pathways of degradation, but rather determine whether the enzymes necessary for metabolic attack of a particular hydrocarbon are produced or active. These sparing effects have a marked influence on the persistence of particular hydrocarbons within a petroleum mixture and thus on the evolution of the weathered petroleum hydrocarbon mixture.

The phenomenon of co-oxidation has been referred to several times in this section. Compounds which otherwise would not be degraded can be enzymatically attacked within the petroleum mixture due to the abilities of the individual microorganisms to grow on other hydrocarbons within the oil (150). A petroleum hydrocarbon mixture, with its multitude of potential primary substrates, provides an excellent chemical environment in which co-oxidation can occur. Many complex branched and cyclic hydrocarbons undoubtedly are removed as environmental contaminants after oil spills as a result of co-oxidation (217, 230, 231). Jamison et al. (155) found that the degradation of hydrocarbons within a high-octane gasoline was not in agreement with the degradation of individual hydrocarbons by pure cultures. They concluded that co-oxidation played a major role in the degradation of the hydrocarbon mixture within gasoline. Horowitz and Atlas (145) found, using chromatographic and mass spectral analysis, that residual oils recovered after exposure in Arctic coastal waters contained similar percentages of the individual components in classes of hydrocarbons regardless of the amount of degradation, indicating that most hydrocarbon components of the oil were being degraded at similar rates. This study is in contrast to most, which show preferential utilization of *n*-alkane hydrocarbons. Co-oxidation was hypothesized to be responsible for the degradation of a number of compounds in the oil to account for the similar rates of disappearance of compounds which are normally easily degraded and those which are normally resistant. Herbes and Schwall (140) also postulated that co-oxidation led to the accumulation of relatively large amounts of partially oxidized products of polynuclear aromatic hydrocarbon degradation in sediments and only limited amounts of CO_2 production. Assessing the role of co-oxidation in natural environments is difficult since multiple microbial populations are present. In the above-mentioned mixed-population studies, synergism could be an alternative hypothesis to explain the observed results. Future studies are needed to clarify the role of co-oxidation in determining the fate of petroleum hydrocarbons in natural ecosystems.

An interesting and as yet unexplained, but consistent, process which occurs during the biodegradation of petroleum hydrocarbons is the enrichment of compounds within the "unresolved envelope" which occurs during gas chromatographic analysis of petroleum hydrocarbons. This envelope is due to a mixture of several compounds which are not resolved into individually defined peaks even by glass capillary gas chromatography. Since these compounds cannot currently be analytically resolved, they cannot be identified. It has been hypothesized that, during the biodegradation of petroleum hydrocarbons, microorganisms are producing (synthesizing) hydrocarbons of different molecular weights or chemical structures. Walker and Colwell (278) found that a wax was produced during microbial degradation of Altamont crude oil but not during abiotic weathering of oil. The high-boiling *n*-alkanes in the wax were associated with microbial degradation of the oil and appeared to be similar to components of tar balls found in the open ocean. The possible production of such high-molecular-weight alkanes during petroleum biodegradation also has been reported by several other investigators (226, 245). The biochemical mechanism for formation of such hydrocarbons during petroleum biodegradation is unknown. Sexstone et al. (251, 252) found that oil biodegradation in tundra soils was accompanied by accumulation of polar lipoidal compounds in the soil column that were not present in fresh oil and were not detected in unoiled soils; the identities of the compounds, however, were not determined. Jobson et al. (158) reported an increase in the polar nitrogen-

sulfur-oxygen fraction during oil biodegradation in soil.

The role of microorganisms in producing complex products from hydrocarbon metabolism which may persist in the environment requires further investigation. Of particular importance is the possible involvement of microorganisms in the formation of tar balls. The synthesis of complex high-molecular-weight hydrocarbons would suggest that microorganisms can play a role in prolonging the impact of petroleum pollutants as well as in abating the impact of such environmental contaminants through biodegradative removal. It is difficult to separate the importance of photochemical and biochemical processes in the formation of oxygenated and polymeric compounds in the environment. It is apparent that the fate of the component hydrocarbons in a mixture of the complexity of petroleum is extremely complicated and requires further research efforts.

TAXONOMIC RELATIONSHIPS OF HYDROCARBON-UTILIZING MICROORGANISMS

The ability to degrade petroleum hydrocarbons is not restricted to a few microbial genera; a diverse group of bacteria and fungi have been shown to have this ability. ZoBell (298) in his review noted that more than 100 species representing 30 microbial genera had been shown to be capable of utilizing hydrocarbons. In a previous review, Bartha and Atlas (34) listed 22 genera of bacteria, 1 algal genus, and 14 genera of fungi which had been demonstrated to contain members which utilize petroleum hydrocarbons; all of these microorganisms had been isolated from an aquatic environment. The most important (based on frequency of isolation) genera of hydrocarbon utilizers in aquatic environments were *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Micrococcus*, *Nocardia*, *Vibrio*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Candida*, *Rhodotorula*, and *Sporobolomyces* (34). Bacteria and yeasts appear to be the prevalent hydrocarbon degraders in aquatic ecosystems. In polluted freshwater ecosystems, bacteria, yeasts, and filamentous fungi all appear to be important hydrocarbon degraders (81). Jones and Eddington (161) found that isolates representing 11 genera of fungi and 6 genera of bacteria were the dominant microbial genera responsible for hydrocarbon oxidation in soil samples. They found that fungi played an important role in the hydrocarbon-oxidizing activities of the soil samples. Cerniglia and Perry (67) found that several fungi (*Penicillium* and *Cunninghamella* spp.) exhibited greater hydrocarbon biodegradation than bacteria (*Flavobacterium*, *Brevibacterium*, and *Arthrobacter* spp.). Recent studies continue to expand the list of microbial species which have been demonstrated to be capable of degrading petroleum hydrocarbons. In one such study, Davies and Westlake (92) examined 60 fungal isolates for their ability to grow on *n*-tetradecane, toluene, naphthalene, and seven crude oils of various compositions. Forty cultures, including 28 soil isolates, could grow on one or more of the crude oils. The genera most frequently isolated from soils were those producing abundant small conidia, e.g., *Penicillium* and *Verticillium* spp. Oil-degrading strains of *Beauveria bassiana*, *Mortierella* spp., *Phoma* spp., *Scolecobasidium obovatum*, and *Tolypocladium inflatum* also were isolated.

Walker et al. (272) compared the abilities of bacteria and fungi to degrade hydrocarbons. The following genera were included in their study: *Candida*, *Sporobolomyces*, *Hansenula*, *Aureobasidium*, *Rhodotorula*, *Cladosporium*, *Penicillium*, *Aspergillus*, *Pseudomonas*, *Vibrio*, *Acinetobacter*, *Leucothrix*, *Nocardia*, and *Rhizobium*. Bacteria and yeasts showed decreasing abilities to degrade alkanes with increasing chain length. Filamentous fungi did not exhibit preferential degradation for particular chain lengths. Patterns of degradation, i.e., which hydrocarbons could be utilized, were similar for bacteria and fungi, but there was considerable variability among individual isolates.

Komagata et al. (177) examined almost 500 yeasts for their ability to degrade hydrocarbons and found 56 that could utilize hydrocarbons, almost all of which were in the genus *Candida*. The fermentation industry has considered using hydrocarbon-utilizing *Candida* species for producing single-cell protein. Ahearn and co-workers (2, 79) have examined yeasts that can utilize hydrocarbons and have isolated strains of *Candida*, *Rhodosporidium*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces*, and *Trichosporon*, which are capable of doing so. *Cladosporium resinae* has been isolated from soil (82, 273) and has repeatedly been found as a contaminant of jet fuels (29, 80, 142, 143). The organism can grow on petroleum hydrocarbons and creates problems in the aircraft industry by clogging fuel lines.

Nyns et al. (210) examined the "taxonomic value" of the property of fungi to assimilate hydrocarbons, i.e., whether the ability of fungi to utilize hydrocarbons was a useful diagnostic test for defining different fungal genera or species. They found that the ability to utilize hydrocarbons occurred mainly in two orders, the *Mucorales* and the *Monilales*. They found that *Aspergillus* and *Penicillium* are rich in hydro-

carbon-assimilating strains. They concluded that the property of assimilating hydrocarbons is relatively rare and that it is a property of individual strains and not necessarily a characteristic of particular species or related taxa. Llanos and Kjoller (187) examined changes in fungal populations in soil after oil waste application. They found that oil application favored growth of *Graphium* and *Paecilomyces*. In their study, strains of *Graphium*, *Fusarium*, *Penicillium*, *Paecilomyces*, *Acremonium*, *Mortierella*, *Gliocladium*, *Trichoderma*, and *Sphaeropsidales* were found to be important groups of soil fungi capable of utilizing crude oil hydrocarbons. In a similar study, Jensen (157) studied the bacterial flora of soil after application of oily waste and found that the most important species of oil degraders belonged to the genera *Arthrobacter* and *Pseudomonas*.

Cundell and Traxler (89) studied 15 isolates from an asphaltic flow near a natural seepage at Cape Simpson, Alaska. The isolates were psychrotrophic and utilized paraffinic, aromatic, and asphaltic petroleum components. The isolates belonged to the bacterial genera *Pseudomonas*, *Brevibacterium*, *Spirillum*, *Xanthomonas*, *Alcaligenes*, and *Arthrobacter*. In northwest Atlantic coastal waters and sediment, Mulkins-Phillips and Stewart (205) reported finding hydrocarbon-utilizing bacteria of the genera *Nocardia*, *Pseudomonas*, *Flavobacterium*, *Vibrio*, and *Achromobacter*.

Walker et al. (285) isolated *Vibrio*, *Pseudomonas*, and *Acinetobacter* species from oil-contaminated sediment and *Pseudomonas* and coryneform species from oil-free sediment. Microorganisms from the oil-free sediment produced greater quantities of polar compounds (asphaltics) after degradation, whereas microorganisms from the oil-contaminated sediment provided greater degradation of saturated and aromatic hydrocarbons. Walker et al. (283) also examined bacteria from water and sediment for their ability to degrade petroleum. Water samples contained a greater variety of bacterial species capable of degrading petroleum than sediment samples. Cultures from both water and sediment contained *Pseudomonas* and *Acinetobacter* species. Bacteria present in the water samples yielded significantly greater degradation of two-, three-, four-, and five-ring cycloalkanes and mono-, di-, tri-, tetra-, and penta-aromatics compared with bacteria from sediment samples.

Both temperature and chemical composition of a crude oil have been shown to have a selective influence on the genera of hydrocarbon utilizers. Cook and Westlake (78) isolated *Achromobacter*, *Alcaligenes*, *Flavobacterium*, and *Cytophaga* at 4°C on a substrate of Prudhoe Bay crude oil; *Acinetobacter*, *Pseudomonas*, and unidentified gram-negative cocci at 4°C on a substrate of Atkinson Point crude oil; *Flavobacterium*, *Cytophaga*, *Pseudomonas*, and *Xanthomonas* at 4°C with Norman Wells crude oil as substrate; and *Alcaligenes* and *Pseudomonas* on Lost Horse crude oil at 4°C. At 30°C, the major genera isolated on Prudhoe Bay crude oil were *Achromobacter*, *Arthrobacter*, and *Pseudomonas*; on Atkinson Point crude oil, the major genera were *Achromobacter*, *Alcaligenes*, and *Xanthomonas*; on Norman Wells crude oil, the major genera were *Acinetobacter*, *Arthrobacter*, *Xanthomonas*, and other gram-negative rods; and on Lost Horse crude oil, they were *Achromobacter*, *Acinetobacter*, and *Pseudomonas*.

Several thermophilic hydrocarbon-utilizing bacteria have been isolated, including species of *Thermomicrobium* and other, yet unidentified genera (200). Both gram-negative and gram-positive thermophilic bacteria have been demonstrated to be capable of hydrocarbon utilization. Some isolated thermophiles are obligate hydrocarbon utilizers and cannot grow on other carbon sources. The possible existence of obligate hydrocarbon utilizers is intriguing but perplexing, since the biochemical degradative pathways indicate that hydrocarbon utilizers must also be capable of metabolizing fatty acids and alcohols.

A large number of *Pseudomonas* species have been isolated which are capable of utilizing petroleum hydrocarbons. The genetics and enzymology of hydrocarbon degradation by *Pseudomonas* species has been extensively studied (70, 71, 104, 116, 294). The genetic information for hydrocarbon degradation in these organisms generally has been found to occur on plasmids. *Pseudomonas* species have been used for genetic engineering, and the first successful test case in the United States to determine whether genetically engineered microorganisms can be patented involved a hydrocarbon-utilizing *Pseudomonas* which was "created" by Chakrabarty (98).

Numerical taxonomy has been used to examine petroleum degrading bacteria (26, 27). Austin et al. (26) examined 99 strains of petroleum-degrading bacteria, isolated from Chesapeake Bay water and sediment, by numerical taxonomy procedures. Eighty-five percent of the petroleum-degrading bacteria examined in this study were defined at the 80 to 85% similarity level within 14 phenetic groups. The groups were identified as actinomycetes (mycelial forms, four clusters), coryneforms, *Enterobacteriaceae*, *Klebsiella aerogenes*, *Micrococcus* spp. (two clusters), *Nocardia* spp. (two clusters), *Pseudomonas* spp. (two clusters), and *Sphaerotilus natans*. It was concluded that degradation of

petroleum is accomplished by a diverse range of bacterial taxa. Of particular note was the finding that some enteric bacteria can utilize petroleum hydrocarbons; the suggestion has been made that some of these enteric bacteria may have acquired this ability through plasmid transfer.

Some cyanobacteria and algae have been found to be capable of hydrocarbon degradation. Walker et al. (282) described a hydrocarbon-utilizing achlorophyllous strain of the alga *Prototheca*. Cerniglia et al. (64) tested nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms for their ability to oxidize naphthalene. They found that *Oscillatoria* spp., *Microcoleus* sp., *Anabaena* spp., *Agmenellum* sp., *Coccochloris* sp., *Nostoc* sp., *Aphanocapsa* sp., *Chlorella* spp., *Dunaliella* sp., *Chlamydomonas* sp., *Ulva* sp., *Cylindretheca* sp., *Amphora* sp., *Porphyridium* sp., and *Petalonia* all were capable of oxidizing naphthalene. Their results indicate that the ability to oxidize aromatic hydrocarbons is widely distributed among the cyanobacteria and algae.

It is now abundantly clear that the ability to utilize hydrocarbons is widely distributed among diverse microbial populations. Hydrocarbons are naturally occurring organic compounds, and it is not surprising that microorganisms have evolved the ability to utilize these compounds. When natural ecosystems are contaminated with petroleum hydrocarbons, the indigenous microbial communities are likely to contain microbial populations of differing taxonomic relationships which are capable of degrading the contaminating hydrocarbons.

DISTRIBUTION OF HYDROCARBON-UTILIZING MICROORGANISMS

Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater, and soil habitats. The literature on actual numbers of hydrocarbon utilizers is confusing because of methodological differences used to enumerate petroleum-degrading microorganisms. A number of investigators have used hydrocarbons incorporated into an agar-based medium (13, 147, 149, 250, 258). This approach has been criticized (9, 74, 202, 277); in some cases, a high correlation has been found between growth on agar media containing hydrocarbons as the sole carbon source and the ability to rigorously demonstrate hydrocarbon utilization of isolates from these media in liquid culture; in other studies, only a low percentage of isolates from agar-based media could be demonstrated to be capable of hydrocarbon utilization. The inclusion of organic contaminants in agar media and the growth of oligotrophic bacteria probably result in the counting of non-hydrocarbon utilizers in some cases when plate counts are used for enumerating hydrocarbon utilizers.

The use of silica gel as a solidifying agent has been shown to improve the reliability of procedures for enumerating hydrocarbon utilizers (246). Walker and Colwell (277) reported that a medium containing 0.5% oil and 0.003% phenol red was best for enumerating petroleum-degrading microorganisms. They also found that addition of Amphotericin B permitted selective isolation of hydrocarbon-utilizing bacteria and that addition of either streptomycin or tetracycline permitted selective isolation of yeasts and fungi. Washing the inoculum to remove contaminating organic compounds did not improve the recovery of petroleum degraders in this study. These authors specifically recommended the use of a silica gel-oil medium for enumerating petroleum-degrading microorganisms; they also suggested that counts of petroleum degraders be expressed as a percentage of the total population rather than as total numbers of petroleum degraders per se.

Buckley et al. (51) characterized the distribution of microorganisms in an estuary relative to ambient hydrocarbon concentrations. Although counts were performed on non-hydrocarbon-based media, at all but two stations most of the species isolated were able to grow on hydrocarbons, indicating that the ability to utilize hydrocarbons is widespread, even in environments not subjected to high levels of hydrocarbon pollution. Crow et al. (86) examined the distribution of hydrocarbon utilizers in surface ocean layers and in the underlying water column. They found that populations of hydrocarbonoclastic microorganisms occurred in concentrations 10 to 100 times greater in the surface layer than at a 10-cm depth.

Mulkins-Phillips and Stewart (205) examined the distribution of hydrocarbon-utilizing bacteria in northwestern Atlantic waters and coastal sediments. The fraction of the total heterotrophic bacteria represented by the hydrocarbon utilizers ranged up to 100%, depending on the area's previous history of oil spillage; most values were less than 10%. They found that the location, numbers, and variety of the microbial hydrocarbon utilizers illustrated their ubiquity and that the broad enzymatic capacity for hydrocarbon degradation indicated the microbial potential for removal or conversion of oil in the environments examined. The presence of hydrocarbon-utilizing microorganisms was demonstrated in sediments and adjacent waters taken from Bermuda, Canadian Northwest Atlantic, and eastern Canadian Arctic marine shorelines.

Bunch and Harland (53) found that numbers

of hydrocarbon utilizers occurred in similar concentrations in Arctic and temperate marine samples; i.e., quantitative differences in the distribution of hydrocarbon utilizers were relatively unimportant over large geographic distances. Indeed, hydrocarbon utilizers have been found to be widely distributed even in cold marine ecosystems (8, 88, 90, 234, 235, 260, 277).

Most-probable-number (MPN) procedures have been suggested as a substitute for plate count procedures for enumerating hydrocarbon-utilizing microorganisms, since such procedures eliminate the need for a solidifying agent and permit direct assessment of the ability to actually utilize hydrocarbons (9, 74). The use of liquid media for MPN procedures permits removal of trace organic contaminants and allows for the chemical definition of a medium with a hydrocarbon as the sole source of carbon. Enumeration methods which incorporate the specificity for counting only hydrocarbon utilizers and which eliminate the problem of counting organisms growing on other trace organic contaminants represents a significant improvement in the accuracy with which numbers of hydrocarbon utilizers can be determined. Higashihara et al. (141) reported that plate counts, using either agar or silica gel solidifying agents, were unsuitable for enumerating hydrocarbon-utilizing microorganisms since many marine bacteria grow and produce microcolonies even on small amounts of organic matter. They recommended the use of an MPN procedure, with hydrocarbons as the source of carbon and trace amounts of yeast extract for necessary growth factors, for accurate enumerations of microbial populations which degrade hydrocarbons in marine environments. Mills et al. (202) compared several media designed for use in an MPN determination of petroleum-degrading microorganisms. The best results, i.e., largest numbers, were obtained with a buffered (32 mM phosphate) liquid medium containing 1% hydrocarbon substrate. In this study, turbidity was used as the criterion for establishing positive results. Counts of petroleum degraders obtained with a liquid medium and an MPN procedure are usually higher than those obtained on silica gel medium with oil added as the carbon source.

^{14}C-radiolabeled hydrocarbons have been used in MPN procedures for determining the distribution of hydrocarbon-utilizing microorganisms. Atlas (9) has described a technique that uses [^{14}C]hexadecane-spiked crude oil to enumerate petroleum-degrading microorganisms. This method uses the conversion of a radiolabeled hydrocarbon to radiolabeled carbon dioxide for establishing positive results in the MPN procedure. Placing the radiolabeled hydrocarbon within a crude oil mimics the availability of hydrocarbons to the microbial community, as would occur in an actual oil spill. Lehmicke et al. (181) used low concentrations of radiolabeled hydrocarbons in MPN determinations; in their studies, the concentrations of radiolabeled hydrocarbons were adjusted to reflect actual concentrations which might be present in soluble form.

Roubal and Atlas (238) studied the distribution of hydrocarbon-utilizing microorganisms in Alaskan Continental Shelf regions, using an MPN procedure based on the mineralization of ^{14}C-labeled hydrocarbons. They reported that hydrocarbon utilizers were ubiquitously distributed, with no significant overall concentration differences between Arctic and subarctic sampling regions nor between surface water and sediment samples. There were, however, significant seasonal differences in numbers of hydrocarbon utilizers. In a study in a temperate region, Raritan Bay, N.J., Atlas and Bartha (13), using oil-agar plate enumerations, also found that numbers of hydrocarbon-utilizing microorganisms were lower during winter than summer. Walker and Colwell (280) similarly found seasonal variations in numbers of hydrocarbon utilizers in Chesapeake Bay.

It is clear from a number of studies that the distribution of hydrocarbon-utilizing microorganisms reflects the historical exposure of the environment to hydrocarbons. A large number of laboratory studies have demonstrated sizable increases in populations of hydrocarbon-utilizing microorganisms when environmental samples are exposed to petroleum hydrocarbons (11, 56, 93, 166, 218, 225, 256, 263, 304).

Mironov (203) and Mironov and Lebed (204) found highly elevated populations of hydrocarbon-utilizing microorganisms in the oil tanker shipping channels of the Indian Ocean and the Black Sea. Polyakova (224) found high numbers of hydrocarbon-oxidizing microorganisms in Neva Bay, U.S.S.R., in association with petroleum inputs. ZoBell and Prokop (305) reported that numbers of hydrocarbon utilizers in sediment of Baritaria Bay, La., were correlated with sources of oil pollutants. Similarly, Atlas and Bartha (13) for Raritan Bay and Colwell et al. (77) and Walker and Colwell (276) for Chesapeake Bay found that distributions of hydrocarbon utilizers correlated highly with sources of oil pollutants entering the bays. The distribution of hydrocarbon utilizers within Cook Inlet was also positively correlated with the occurrence of hydrocarbons in the environment (238). LePetit et al. (185) reported that bacteria utilizing a gas-oil as the sole carbon source represented 10% of the heterotrophic bacteria in the area of a refinery

effluent compared with 4% in an area not directly polluted by hydrocarbons. The degradation potential was highest in areas of chronic discharge (261).

Several studies have shown a rise in populations of hydrocarbon-utilizing microorganisms after oil spills. Kator and Herwig (167) found that, within a few days after spillage of South Louisiana crude oil in a coastal estuary in Virginia, levels of petroleum-degrading bacteria rose by several orders of magnitude. The elevated levels of hydrocarbon utilizers were maintained for over 1 year. Raymond et al. (229) found significant increases in hydrocarbon-utilizing microorganisms in soils receiving hydrocarbons; increased populations were maintained throughout the year. Pinholt et al. (221) examined the microbial changes during oil decomposition in soil. They found an increase from 60 to 82% in oil-utilizing fungi and an increase from 3 to 50% in oil-degrading bacteria after a fuel oil spill. Oppenheimer et al. (214) found a tendency toward higher ratios of hydrocarbon-utilizing bacteria to total viable heterotrophs in the active Ekofisk oil field of the North Sea, probably due to the occurrence of hydrocarbons in the sediments of this region. Gunkel et al. (135) confirmed the occurrence of high numbers of hydrocarbon-utilizing microorganisms in the vicinity of the North Sea oil fields and found a high correlation between concentrations of hydrocarbons and oil-utilizing bacteria in the North Sea.

High numbers of fungi have been found in association with the Cape Simpson, Alaska, oil seeps (31). Numbers of filamentous fungi 0.2 m from the edge of the seep were reported to be three times higher than those 50 m from the seep; bacterial populations in ponds in contact with the Cape Simpson oil seeps were found to be higher than in unstressed ponds; bacterial populations in soils adjacent to the asphaltic sections of the seeps were higher than those 50 m from the seep.

In experimental field studies in the Arctic, Atlas and co-workers have found large increases in hydrocarbon-utilizing microorganisms in marine (8, 20, 21, 24, 25, 147), freshwater (25, 146), and soil (250, 253) ecosystems; concentrations of hydrocarbon-utilizing microorganisms have been found to rise rapidly and dramatically in response to acute inputs of petroleum hydrocarbons. Bergstein and Vestal (38), however, found a lack of elevated microbial populations in an oil-treated tundra pond unless phosphate also was added. Horowitz and Atlas (146), using a continuous-flow-through model system, found large increases and shifts to a high percentage of hydrocarbon utilizers in Arctic coastal water when nitrogen and phosphorus were added to oil slicks. Sexstone and Atlas (250) found that addition of crude oil to Arctic tundra soils resulted in large increases in total numbers of heterotrophs and of oil-utilizing microorganisms. The response of microbial populations to contaminating oil was found to depend on soil type and depth. Increases in microbial populations in subsurface soils paralleled downward migration of the oil (249).

Sparrow et al. (257) found a rise in oil-utilizing bacterial populations in taiga soils which were experimentally contaminated with hot Prudhoe Bay crude oil. Studies in the Swan Hills area of north-central Alberta, Canada, by Cook and Westlake (78) showed slightly increased bacterial populations 308 and 433 days after treatment with Swan Hills oil at an application rate of 6.5 liters/m^2. Increases in numbers of bacteria were significantly higher when the plots were also treated with urea-phosphate fertilizer. Similar results were obtained at Norman Wells 321 and 416 days after treatment with 6.5 liters of Norman Wells crude per m^2. As with the Swan Hills spill, slight increases in bacterial numbers occurred when oil alone was added, and significantly higher increases occurred when fertilizer was also added.

Gunkel (133, 134) reported that populations of hydrocarbon utilizers were elevated in sediments affected by the Torrey Canyon spill. Stewart and Marks (258) found higher numbers of hydrocarbon utilizers in sediment affected by the Arrow spill in Chedabucto Bay, Nova Scotia; 5 years after the spill, only a few sites examined had significant concentrations of residual petroleum and elevated counts of hydrocarbon utilizers. Significantly elevated numbers of hydrocarbon utilizers (several orders of magnitude above normal) were found after the Amoco Cadiz spill in Brittany (19) and the XTOC-I well blowout in the Bay of Campeche, Gulf of Mexico (23). In the case of the Amoco Cadiz spill, the numbers of hydrocarbon utilizers in intertidal sediments were positively correlated with the degree of hydrocarbon contamination; during recovery after the spillage, the numbers of hydrocarbon utilizers returned at most sites to background levels as the oil disappeared due to biodegradative removal. Counts of hydrocarbon utilizers associated with an oil-in-water emulsion (mousse) from the XTOC-I well blowout were three to five orders of magnitude higher than in surface water samples not contaminated with oil (23). In the sediment of an Arctic lake that had been contaminated with a leaded refined gasoline, populations of hydrocarbon-utilizing microorganisms were found to be significantly elevated within a few hours of the spill (146) through 1 year after the spill (147). This degree

of elevation in numbers of microbial hydrocarbon utilizers corresponded with the degree of contamination.

In general, population levels of hydrocarbon utilizers and their proportions within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons. In unpolluted ecosystems, hydrocarbon utilizers generally constitute less than 0.1% of the microbial community; in oil-polluted ecosystems, they can constitute up to 100% of the viable microorganisms. The degree of elevation above unpolluted compared reference sites appears to quantitatively reflect the degree or extent of exposure of that ecosystem to hydrocarbon contaminants.

ENVIRONMENTAL FACTORS INFLUENCING BIODEGRADATION OF PETROLEUM HYDROCARBONS

The fate of petroleum hydrocarbons in the environment is largely determined by abiotic factors which influence the weathering, including biodegradation of the oil. Factors which influence rates of microbial growth and enzymatic activities affect the rates of petroleum hydrocarbon biodegradation. The persistence of petroleum pollutants depends on the quantity and quality of the hydrocarbon mixture and on the properties of the affected ecosystem. In one environment petroleum hydrocarbons can persist indefinitely, whereas under another set of conditions the same hydrocarbons can be completely biodegraded within a relatively few hours or days.

PHYSICAL STATE OF OIL POLLUTANTS

The physical state of petroleum hydrocarbons has a marked effect on their biodegradation. At very low concentrations hydrocarbons are soluble in water, but most oil spill incidents release petroleum hydrocarbons in concentrations far in excess of the solubility limits (46, 115, 139, 195). The degree of spreading determines in part the surface area of oil available for microbial colonization by hydrocarbon-degrading microorganisms; in aquatic systems, the oil normally spreads, forming a thin slick (39). The degree of spreading is reduced at low temperatures because of the viscosity of the oil. In soils, petroleum hydrocarbons are absorbed by plant matter and soil particles, limiting its spreading.

Wodzinsky and LaRocca (295) found that liquid aromatic hydrocarbons were utilized by bacteria at the water-hydrocarbon interface but that solid aromatic hydrocarbons were not metabolized. At 30°C diphenylmethane is a liquid and could be degraded, but at 20°C the solid form of diphenylmethane could not be utilized by a *Pseudomonas* sp. They also found that naphthalene could not be utilized in the solid form but could be utilized if dissolved in a liquid hydrocarbon. Atlas (unpublished data) similarly found that hexadecane supported only marginal bacterial growth at 5°C when the compound was in the solid form, but if hexadecane was dissolved in another liquid hydrocarbon or crude oil, extensive degradation of the liquid hexadecane occurred at 5°C. The role of temperature in determining the physical state of a hydrocarbon and the influence of the physical state on rates of microbial hydrocarbon degradation are apparent in these studies.

Hydrocarbon-degrading microorganisms act mainly at the oil-water interface. Hydrocarbon-degrading microorganisms can be observed growing over the entire surface of an oil droplet; growth does not appear to occur within oil droplets in the absence of entrained water. Availability of increased surface area should accelerate biodegradation (117, 118). Not only is the oil made more readily available to microorganisms, but movement of emulsion droplets through a water column makes oxygen and nutrients more readily available to microorganisms.

In aquatic ecosystems, oil normally forms emulsions. This has been termed "pseudosolubilization" of the oil (136). The water-in-oil emulsion which occurs in seawater after oil spills is referred to as "chocolate mousse" or simply "mousse." The processes involved in the formation of mousse have been examined by a number of investigators (40, 54, 95). Mousse is chemically and physically heterogeneous. Photooxidation (54) and microbial oxidation (40) have been reported to play a role in mousse formation under different environmental conditions; both abiotic and microbial processes appear to be capable of initiating mousse formation under appropriate environmental conditions. In some cases, a fine emulsion is formed with small droplets of mousse. In these cases, the hydrocarbons in the mousse probably are more susceptible to microbial degradation, and their fate is similar to that of "dissolved" hydrocarbons. Mousse can also refer to large accumulations of emulsified oil in "globs" up to 1 m in diameter. Such large "mousse plates" have limited surface areas, and hydrocarbons internal to the mousse may be spared from microbial degradation. Davis and Gibbs (94) found that large accumulations of "mousse" weathered extremely slowly with no net loss of hydrocarbons over 2 years. Atlas et al. (23) proposed that degradation of hydrocarbons released into the Gulf of Mexico by the XTOC-I blowout was limited in part by the physical properties of the mousse accumula-

tions. Colwell et al. (75) postulated that degradation of oil from the Metula spill was restricted by the formation of tar balls and aggregates of oil which restricted accessibility of the hydrocarbons to microorganisms. Microbial degradation was ineffective when oil was deposited on the beach and subsequently buried or when the oil formed asphalt layers or tar balls.

Dispersants have been used to treat oil spills. In some cases the use of toxic dispersants probably has resulted in greater ecological impact than the oil spill itself; such was the case in the Torrey Canyon incident (83, 255). Some dispersants may contain chemicals which are inhibitory to microorganisms. Without toxicity, however, dispersion can enhance petroleum biodegradation. Mulkins-Phillips and Stewart (207) found that some dispersants enhanced *n*-alkane degradation in crude oil, but that other dispersants had no effect. Gatellier et al. (117, 118) and Robichaux and Myrick (236) likewise found that some dispersants inhibited hydrocarbon-oxidizing populations, whereas others enhanced hydrocarbon-degrading microorganisms. Atlas and Bartha (14) tested several dispersants and found that all increased the rate but not the extent of hydrocarbon mineralization.

A number of hydrocarbon-degrading microorganisms produce emulsifying agents (1, 131, 233, 298). Some of these bioemulsifiers have been considered for use in cleaning oil storage tanks, such as on supertankers (136). Reisfeld et al. (233) have studied an *Arthrobacter* strain which extensively emulsifies oil when growing on hydrocarbons. Zajic and co-workers (297) have characterized the emulsifying agents produced by strains of *Pseudomonas* and *Corynebacterium*. In some cases, the emulsifying agents appear to be fatty acids or derivatives of fatty acids; in other cases, more complex polymers are the active emulsifying agents. Although the production of emulsifying agents should increase the susceptibility of hydrocarbons in an oil to microbial degradation, microbial strains which effectively emulsify oil often do not extensively degrade the hydrocarbons in the oil. It is not clear yet why extensive emulsification does not permit greater hydrocarbon degradation by these organisms.

After extensive weathering, petroleum hydrocarbons often occur in the environment as tar balls. Hydrocarbons in tar are quite resistant to microbial degradation. Many of the hydrocarbons in tar have chemical structures which are not readily attacked by microbial enzymes. The surface area-to-volume ratio of a tar ball is not favorable for microbial growth on this insoluble substrate. Tar balls often accumulate on beaches where microbial activities are limited by available water, which is needed to support microbial growth and enzymatic hydrocarbon-degrading activities.

From the point of view of microbial hydrocarbon degradation, dissolution and emulsification of hydrocarbons appear to have a positive effect on degradation rates. If there are no adverse toxic effects, dispersion of oil should accelerate microbial hydrocarbon degradation. This is an important consideration when determining whether dispersants should be added to oil spills. Increased toxicity must remain, however, a major concern when considering the use of such chemical dispersants.

TEMPERATURE

Hydrocarbon biodegradation can occur over a wide range of temperatures, and psychrotrophic, mesophilic, and thermophilic hydrocarbon-utilizing microorganisms have been isolated. ZoBell (303) and Traxler (263) reported on hydrocarbon degradation at below 0°C; Klug and Markovetz (174, 175) and Mateles et al. (192) reported on hydrocarbon degradation at 70°C.

Temperature can have a marked effect on the rates of hydrocarbon degradation. The effects of temperature on the physical state of hydrocarbons was discussed in the previous section. ZoBell (301) found that hydrocarbon degradation was over an order of magnitude faster at 25°C than at 5°C. Very low rates of hydrocarbon utilization were found by Gunkel (132) at low water temperatures. Ludzack and Kinkead (189) found that motor oil was rapidly oxidized at 20°C but not at 5°C. Mulkins-Phillips and Stewart (206) found that, 9 months after the spillage of Bunker C fuel oil into Chedabucto Bay, the bacterial populations isolated from contaminated areas showed rates of degradation at 5°C that were 21 to 70% less during 14 days of incubation than during 7 days at 10°C.

There are seasonal shifts in the composition of the microbial community which can be reflected in the rates of hydrocarbon metabolism at a given temperature. Atlas and Bartha (13) found that higher numbers of hydrocarbon utilizers capable of growth at 5°C were present in Raritan Bay, N.J., during winter than during other seasons. Rates of hydrocarbon mineralization measured at 5°C were significantly higher in water samples collected in winter than in summer. The evidence suggests a seasonal shift to a microbial community capable of low-temperature hydrocarbon degradation.

Gibbs and Davis (120) studied the degradation of oil in beach gravel at temperatures from 6 to 26°C. They found a Q_{10} (6 to 16°C) of 3.3 and a Q_{10} (11 to 21°C) of 2.05. The average Q_{10} value

of 2.7 was the same as the value found in other studies, by Gibbs et al. (121), on the effects of temperature on the degradation of oil in seawater. Atlas and Bartha (10) found a Q_{10} of approximately 4, using seawater over a temperature range of 5 to 20°C.

Atlas and Bartha (10) found that the effects of temperature differ, depending on the hydrocarbon composition of a petroleum mixture. Low temperatures retard the rates of volatilization of low-molecular-weight hydrocarbons, some of which are toxic to microorganisms. The presence of such toxic components was found to delay the onset of oil biodegradation at low temperatures (10). In a subsequent study, Atlas (5) examined the biodegradability of seven different crude oils and found biodegradation to be highly dependent on the composition and on incubation temperature. At 20°C, lighter oils had greater abiotic losses and were more susceptible to biodegradation than heavier oils; rates of oil mineralization for the heavier oils were significantly lower at 20°C than for the lighter ones. The light crude oils, however, had toxic volatile components which evaporated only slowly, inhibiting microbial degradation of these oils at 10°C. A significant lag phase before the onset of hydrocarbon biodegradation was found for the lighter oils. No toxic volatile fraction was associated with the heavy oils tested. Paraffinic, aromatic, and asphaltic fractions were subject to biodegradation. Some preference was shown for paraffin degradation, especially at low temperatures. Horowitz and Atlas (145) found that during summer, in Arctic surface waters, different structural classes of hydrocarbons were degraded at similar rates. They postulated that at low temperatures cometabolism played an important role in determining the rates of disappearance of hydrocarbons in the mixture.

Walker and Colwell (274), using a model petroleum incubated with estuarine water collected during winter, found that slower but more extensive biodegradation occurred at 0°C than at higher temperatures. Decreased toxicity of hydrocarbons at lower temperatures was hypothesized to explain the more extensive growth at the lower temperature.

Ward and Brock (289) studied the influence of environmental factors on the rates of hydrocarbon oxidation in temperate lakes. Rates of hydrocarbon oxidation were assessed by using the conversion of ^{14}C-radiolabeled hexadecane to $^{14}CO_2$. They found that a lag phase preceded hydrocarbon oxidation and that the length of the lag phase depended on population density or on factors influencing growth rate. Hydrocarbon oxidation was coincident with growth and was presumed to occur only under conditions of development of indigenous hydrocarbon-degrading microorganisms. They found that hydrocarbon-degrading microorganisms persisted during the year, but that there were seasonal variations in the rates of hydrocarbon oxidation. Rates of petroleum hydrocarbon biodegradation were correlated with temperature. During winter, spring, and fall, temperature was a major limiting factor. Dibble and Bartha (102) found that the rates of disappearance of hydrocarbons from an oil-contaminated field in New Jersey showed a definite correlation with mean monthly temperature.

Arhelger et al. (4) compared Arctic and subarctic hydrocarbon biodegradation. In situ [^{14}C]dodecane oxidation rates based on $^{14}CO_2$ production were: Port Valdez, 0.7 g/liter per day; Chukchi Sea, 0.5 g/liter per day; and Arctic Ocean, 0.001 g/liter per day. This study indicates that rates of hydrocarbon degradation show a definite climatic shift and are lower in the Arctic Ocean than in more southerly Alaskan regions.

Atlas et al. (7, 21) examined the degradation of Prudhoe Bay crude oil in Arctic marine ice, water, and sediment ecosystems. Petroleum hydrocarbons were degraded slowly. They found that ice greatly restricted losses of light hydrocarbons and that biodegradation of oil on the surface of ice or under sea ice was negligible. They concluded that petroleum hydrocarbons will remain in cold Arctic ecosystems for long periods of time after oil contamination. In these studies, however, temperature was not specifically elucidated as a major factor limiting hydrocarbon degradation, except as it related to the occurrence of ice.

Colwell et al. (75) reported greater degradation of Metula crude oil at 3°C than at 22°C with mixed microbial cultures in beach sand samples; when 0.1% oil was added, 48% of the added hydrocarbons were degraded at an incubation temperature of 3°C, compared with only 21% degraded at 22°C with cultures adapted at the same temperatures as the incubation temperature. They found that under in situ conditions oil degradation proceeded slowly, but concluded that temperature does not seem to be the limiting factor for petroleum degradation in the Antarctic marine ecosystem affected by the Metula spill.

A number of studies have been conducted on the fate of oil in cold Arctic soils. Sexstone and colleagues (251–253) have reported very long persistence times for oil in tundra soils. It appears that degradation of hydrocarbons ceases during winter when tundra soils are frozen. Westlake and colleagues (78, 158, 292) found that the microbial populations in northern soils were able to degrade hydrocarbons at the am-

bient temperatures found during the warmer seasons. Several aspects of these studies were discussed earlier in this review.

It is apparent that the influence of temperature on hydrocarbon degradation is more complex than simple consideration of Q_{10} values. The effects of temperature are interactive with other factors, such as the quality of the hydrocarbon mixture and the composition of the microbial community. Hydrocarbon biodegradation can occur at the low temperatures (<5°C) that characterize most of the ecosystems which are likely to be contaminated by oil spills. Temperature often is not the major limiting factor for hydrocarbon degradation in the environment except as it relates to other factors such as the physical state of the oil or whether liquid water is available for microbial growth. Concern must be expressed, however, regarding the rates of microbial oil degradation in Arctic and subarctic regions. These are areas of new petroleum development and the data gathered to date suggest that rates of microbial degradation in these cold ecosystems may not be adequate to rapidly remove hydrocarbon contaminants.

NUTRIENTS

There is some confusion and considerable apparent conflict in the literature regarding the limitation of petroleum biodegradation by available concentrations of nitrogen and phosphorus in seawater. Several investigators (12, 32, 109, 111, 132, 183, 184) have reported that concentrations of available nitrogen and phosphorus in seawater are severely limiting to microbial hydrocarbon degradation. Other investigators (173), however, have reached the opposite conclusion, i.e., that nitrogen and phosphorus are not limiting in seawater. The difference in results is paradoxical and appears to be based on whether the studies are aimed at assessing the biodegradation of hydrocarbons within an oil slick or the biodegradation of soluble hydrocarbons. When considering an oil slick, there is a mass of carbon available for microbial growth within a limited area. Since microorganisms require nitrogen and phosphorus for incorporation into biomass, the availability of these nutrients within the same area as the hydrocarbons is critical. Extensive mixing can occur in turbulent seas, but in many cases the supply of nitrogen and phosphorus is dependent on diffusion to the oil slick. Rates of diffusion may be inadequate to supply sufficient nitrogen and phosphorus to establish optimal C/N and C/P ratios for microbial growth and metabolism. Researchers examining the fate of large oil spills have thus properly concluded in many cases that concentrations of N and P are limiting with respect to rates of hydrocarbon biodegradation. When considering soluble hydrocarbons, nitrogen and phosphorus are probably not limiting since the solubility of the hydrocarbons is so low as to preclude establishment of an unfavorable C/N or C/P ratio. Investigators considering the fate of low-level discharges of hydrocarbons (soluble hydrocarbons) have, thus, properly concluded that available nutrient concentrations are adequate to support hydrocarbon biodegradation.

Floodgate (111), in considering the limitations of nutrients to biodegradation of hydrocarbons in the sea, proposed the concept of determining the "nitrogen demand," analogous to the concept of biochemical oxygen demand. Based on Kuwait crude oil at 14°C, the nitrogen demand was found to be 4 nmol of nitrogen per ng of oil. Bridie and Bos (47) found that addition of 3.2 mg of ammonium nitrogen and 0.6 mg of phosphate permitted maximal rates of degradation of Kuwait crude in seawater at a concentration of 70 mg of oil per liter. Atlas and Bartha (12) found that concentrations of 1 mg of nitrogen and 0.07 mg of phosphorus per liter supported maximal degradation of Sweden crude oil in New Jersey coastal seawater at a concentration of 8 g of oil per liter. Reisfeld et al. (233) reported optimal concentrations of nitrogen and phosphorus of 11 and 2 mg per liter for biodegradation of 1 g of Iranian crude oil per liter in Mediterranean seawater.

Colwell et al. (75) concluded that Metula oil is degraded slowly in the marine environment, most probably because of limitations imposed by the relatively low concentrations of nitrogen and phosphorus available in seawater.

Ward and Brock (289) reported that although temperature was the main limiting factor much of the year, during summer nutrient deficiencies limited oil biodegradation in temperate lakes. Higher rates of oil biodegradation could be obtained by addition of nitrogen and phosphorus. High rates of hydrocarbon degradation were found only during 1 month of the year when temperature and nutrient supplies were optimal. They concluded that environmental factors limited hydrocarbon biodegradation, but that availability of hydrocarbon-utilizing microorganisms within the indigenous microbial community was not a limiting factor.

LePetit and N'Guyen (184) found that the artificial stimulation of bacterial hydrocarbon degradation requires the addition of phosphorus to seawater. They reported optimal concentrations of phosphorus to support hydrocarbon degradation of between 2×10^{-4} and 8×10^{-4} M for seawater and between 1.5×10^{-3} and 3×10^{-3} M for coastal waters receiving a significant supply of fresh water. Inhibition of bacterial development was observed with higher phosphate con-

centrations. Gibbs (119) calculated that 1 m^3 of Irish Sea water provides sufficient nitrogen to degrade 30 g of oil per year at summer temperatures and 11 g of oil per year at winter temperatures.

Bergstein and Vestal (38) studied the biodegradation of crude oil in Arctic tundra ponds. They concluded that oleophilic fertilizer may provide a useful tool to enhance the biodegradation of crude oil spilled on such oligotrophic waters. Without addition of nitrogen and phosphorus, hydrocarbon biodegradation was limited. Atlas and Bartha (17) described an oleophilic nitrogen and phosphorus fertilizer which could overcome limitations of nitrogen and phosphorus in seawater and stimulate petroleum biodegradation in seawater. The fertilizer consisting of paraffinized urea and octylphosphate supported degradation of oil in seawater. Optimal C/N and C/P ratios were 10:1 and 100:1, respectively. In conjunction with the U.S. Office of Naval Research, they obtained a patent for use of fertilizers for stimulating oil degradation in seawater (33).

Olivieri et al. (212) described a slow-release fertilizer containing paraffin-supported magnesium ammonium phosphate as the active ingredient for stimulating petroleum biodegradation. They reported that the biodegradation of Sarir crude oil in seawater was considerably enhanced by addition of the paraffin-supported fertilizer. After 21 days, 63% of the oil had disappeared when fertilizer was added compared with 40% in a control area. Kator et al. (168) suggested the use of paraffinized ammonium and phosphate salts for enhancing oil biodegradation in seawater. Raymond et al. (232) were able to stimulate the microbial degradation of hydrocarbons in contaminated groundwaters by procedures which included the addition of nitrogen and phosphorus nutrients.

Dibble and Bartha (99) examined the effect of iron on the biodegradation of petroleum in seawater. Biodegradation of south Louisiana crude oil and the effects of nitrogen, phosphorus, and iron supplements on this process were compared in polluted and relatively clean littoral seawater collected along the New Jersey coast. Without supplements, the biodegradation of south Louisiana crude oil was negligible in both seawater samples. Addition of nitrogen and phosphorus allowed very rapid biodegradation; up to 73% of the oil was degraded within 3 days in polluted seawater. Total iron in the seawater sample was high (5.2 mM), and the addition of iron did not increase biodegradation rates. In less polluted and less iron-rich (1.2 mM Fe) seawater samples, biodegradation of south Louisiana crude oil was considerably slower (21% in 3 days), and addition of chelated iron had a stimulating effect. Ferric octoate was shown to have a stimulating effect on south Louisiana crude oil biodegradation, similar to that of chelated iron. Ferric octoate in combination with paraffinized urea and octylphosphate is suitable for treatment of floating oil slicks. The authors concluded that spills of south Louisiana crude oil and similar oils can be cleaned up rapidly and efficiently by stimulated biodegradation, provided that water temperatures are favorable.

Dibble and Bartha (100) examined the effect of environmental factors on the biodegradation of oil sludge. They conducted a laboratory study aimed at evaluating and optimizing the environmental factors of land farming, i.e., disposal by biodegradation in soil of oily sludges generated in the refining of crude oil. They found that oil sludge biodegradation was optimal at a soil water-holding capacity of 30 to 90%, a pH of 7.5 to 7.8, a C/N ratio of 60:1, and a C/P ratio of 800:1. Optimal temperatures were 20°C or above. They reported that an application rate of 5% (by weight) oil sludge hydrocarbon to soil, i.e., 100,000 liters/hectare, gave a good compromise between high biodegradation rates and efficient land use and resulted in the best overall biodegradation rate of oil hydrocarbon classes. Frequent small applications resulted in higher biodegradation rates than single large applications.

Fedorak and Westlake (personal communication) found that, without added nutrients, aromatic hydrocarbons were more readily attacked than saturated hydrocarbons by soil and marine microbes; addition of nitrogen and phosphorus nutrients stimulated degradation of saturated hydrocarbons more than of aromatic hydrocarbons.

Westlake et al. (292) examined the in situ degradation of oil in a soil of the boreal region of the northwest territories of Canada. Where fertilizer containing nitrogen and phosphorus was applied to the oil, there was a rapid increase in bacterial numbers, but no increase in fungal propagules. This was followed by a rapid disappearance of *n*-alkanes and isoprenoids and a continuous loss of weight of saturated compounds in the recovered oil. The seeding of oil slick plots with oil-degrading bacteria had no effect on the composition of the recovered oil. Jobson et al. (159) similarly found that nitrogen and phosphorus addition stimulated hydrocarbon degradation in oil applied to soil but that seeding did not stimulate degradation. Hunt et al. (152) found that fertilizer application to subarctic soils enhanced microbial hydrocarbon degradation. They found, however, in laboratory tests that nitrogen addition caused an initial negative response in microbial activity which was followed by enhanced biodegradation; mi-

crobial activity also responded positively to phosphorus addition.

Raymond et al. (229) studied oil biodegradation in soil. Greater oil degradation was found in soils receiving fertilizer application and rototilling than in untreated soils. They did not find any leaching of hydrocarbons into groundwater. Dibble and Bartha (101) studied the leaching aspect of oil sludge biodegradation in soil. They added fertilizer to oil sludges in soils and examined the leachate for phosphate and undegraded hydrocarbons. There was a modest increase in total organic carbon in the leachate, presumably due to hydrocarbon biodegradation, but no undegraded hydrocarbons or phosphorus was recovered in the leachate. The results support the concept that oil sludge application to soil can be used for biodegradative removal of these materials.

The above studies indicate that the available concentrations of nitrogen and phosphorus severely limit the extent of hydrocarbon degradation after most major oil spills. Rates of nutrient replenishment generally are inadequate to support rapid biodegradation of large quantities of oil. The addition of nitrogen- and phosphorus-containing fertilizers can be used to stimulate microbial hydrocarbon degradation.

OXYGEN

As with nutrients, there has been controversy over whether oxygen is absolutely required for hydrocarbon biodegradation or whether hydrocarbons are subject to anaerobic degradation. The current evidence supports the view that anaerobic degradation by microorganisms at best proceeds at negligible rates in nature (28, 288). The existence of microorganisms which are capable of anaerobic hydrocarbon metabolism has not, however, been excluded. In fact, there have been several reports of isolated microorganisms which are capable of alkane dehydrogenation (72, 153, 215, 247, 264) under anaerobic conditions. These organisms have an enzymatic mechanism which should permit addition of water across the double bond, forming a secondary alcohol, and therefore permit anaerobic growth. Although there have been preliminary reports (264) on the ability of isolated organisms to grow on *n*-alkanes anaerobically, these findings generally have not been adequately repeated upon further testing (R. W. Traxler, personal communication). In the case of the *Pseudomonas* strain studied by Senez and Azoulay (247), the organisms consumed oxygen when growing on heptane even though it had an *n*-heptane dehydrogenase enzyme.

There have been few reports on the anaerobic degradation of hydrocarbons in natural ecosystems (28, 48, 49, 220, 305). These reports suggested that nitrate or sulfate could serve as an alternate electron acceptor during anaerobic respiration using hydrocarbon substrates. This mechanism has not been biochemically confirmed for hydrocarbon utilization in pure cultures, and the criteria used for assessing anaerobic hydrocarbon degradation in the above-mentioned studies generally were inadequate to establish definitive results; either there was a lack of exhaustive evidence for the complete exclusion of oxygen or there was a lack of chemical evidence needed to establish that hydrocarbons were in fact degraded. In the study by Shelton and Hunter (254), there was an 11% decrease in hexane-extractable material under anaerobic conditions compared with only 4% under aerobic conditions in oiled sediments during 30 weeks of incubation. They concluded, however, that the rapid loss of aliphatic hydrocarbons under anaerobic conditions could not be accounted for by microbial degradation.

Hambrick et al. (138) found that, at pH values between 5 and 8, mineralization of hydrocarbons in estuarine sediments was highly dependent on oxygen availability. Rates of hydrocarbon degradation decreased with decreasing oxygen reduction potential, i.e., with increasing anaerobiosis. They concluded that hydrocarbons would persist in reduced sediments for longer periods of time than would hydrocarbon contaminants in aerated surface layers. Some mineralization of alkanes (about 10 to 20%) was reported during 35 days of incubation under anaerobic conditions, but mineralization of naphthalene was insignificant (about 0.4% under these incubation conditions). Naphthalene mineralization increased from 0.6 to 22.6% when the redox potential was gradually increased from −220 mV to +130 mV over an additional 35-day incubation period (97). Ward and Brock (290) similarly found that hexadecane was rapidly mineralized in freshwater lake sediments under aerobic conditions but that almost no hydrocarbon mineralization occurred under anaerobic conditions. Addition of nitrate and sulfate, in this study, failed to increase hydrocarbon mineralization under anaerobic conditions.

In a recent study, Ward et al. (288) compared rates of hydrocarbon oxidation in sediments affected by the Amoco Cadiz spillage under aerobic and anaerobic conditions. With ^{14}C-labeled hydrocarbons, $^{14}CO_2$ production from heptadecane and toluene, but not from hexadecane, was found during anaerobic incubation. Methanogenesis could be demonstrated in these tests, indicating rigorous anaerobic conditions. Although measurable degradation rates under anaerobic conditions were found, rates of $^{14}CO_2$

production were orders of magnitude lower under anaerobic than under aerobic conditions. In the absence of oxygen, less than 5% of added hydrocarbon was oxidized to $^{14}CO_2$ during 233 days compared with over 20% during 14 days under aerobic conditions. In this study, petroleum was found in a relatively unweathered state in anaerobic sediments oiled by the Amoco Cadiz spill, indicating that hydrocarbons are indeed preserved from microbial attack under anaerobic conditions in the environment.

The importance of oxygen for hydrocarbon degradation is indicated by the fact that the major degradative pathways for both saturated and aromatic hydrocarbons, discussed earlier, involve oxygenases and molecular oxygen. The theoretical oxygen demand is 3.5 g of oil oxidized per g of oxygen (111, 301). ZoBell (301) calculated that the dissolved oxygen in 3.2×10^5 liters of seawater therefore would be required for the complete oxidation of 1 liter of oil. Within anoxic basins, the hypolimnion of stratified lakes and benthic sediments, oxygen may severely limit biodegradation.

Johnston (160) examined the consumption of oxygen in sand columns containing Kuwait crude oil. The oxygen concentration in the interstitial water decreased rapidly. The mean rate of oxygen consumption over 4 months was 0.45 g/m^2 per day at 10°C, corresponding to an oil degradation rate of 90 mg of oil/m^2 per day. Biodegradation of oil in sediments has been found to be stimulated by bioturbation (128, 178). The introduction of oxygen by burrowing animals such as polychaete worms is apparently very important in determining the rate of biodegradation of hydrocarbons in oil-contaminated sediments.

Jamison et al. (154) used forced aeration to supply oxygen for hydrocarbon biodegradation in a groundwater supply which had been contaminated by gasoline. Nutrient addition without aeration failed to stimulate biodegradation, but when both nutrients and oxygen were supplied, it was estimated that up to 1,000 barrels of gasoline was removed by stimulated microbial degradation. Such manipulations to supply oxygen probably are not feasible in open systems where natural forces such as wind and wave action will have to be relied upon for turbulent mixing and resupply of oxygen to support biodegradation of oil.

Regardless of whether hydrocarbon degradation can occur at all under anaerobic conditions, the environmental importance of anaerobic hydrocarbon biodegradation can be discounted. Rapid biodegradation of hydrocarbons does not occur in anaerobic environments. Hydrocarbons which enter anaerobic environments such as anoxic sediments are well preserved and persist indefinitely as environmental contaminants.

SALINITY AND PRESSURE

The influence of several other environmental factors on hydrocarbon biodegradation has been studied. Typically these factors are specific features of particular ecosystems such as saline lakes or deep seas (high hydrostatic pressure), which represent specialized environments that may be contaminated by petroleum hydrocarbons.

Ward and Brock (291) examined hydrocarbon biodegradation in hypersaline environments. When hydrocarbons were added to natural samples of various salinities (from 3.3 to 28.4%) from salt evaporation ponds of Great Salt Lake, Utah, rates of metabolism of these compounds decreased as salinity increased. Rate limitations did not appear to relate to low oxygen levels or to availability of organic nutrient. Gas chromatographic examination of hexane-soluble components of tar samples from natural seeps at Rozel Point in Great Salt Lake demonstrated no evidence of biological oxidation of isoprenoid alkanes which are subject to degradation in normal environments. Attempts to enrich for microorganisms, in saline waters, able to use mineral oil as a sole source of carbon and energy were successful below, but not above, approximately 20% salinity. The study strongly suggests a general reduction of metabolic rate at extreme salinities and raises doubts about the biodegradation of hydrocarbons in hypersaline environments.

Hydrocarbon pollutants in the world's oceans may eventually sink; some petroleum components may contaminate deep benthic zones. Microbial degradation of organic matter in the deep sea has been found to be greatly restricted (156). Hydrocarbons do not appear to be an exception. Schwarz et al. (242–244) examined the growth and utilization of hydrocarbons at ambient and in situ pressure for deep-sea bacteria. The rate of hydrocarbon utilization under high pressure and ambient temperatures was found to be significantly less than rates found under conditions of ambient temperatures and atmospheric pressure. Whereas 94% of hexadecane was utilized within 8 weeks at 1 bar, at 500 bars it took 40 weeks for similar degradation. It appears that oil which enters deep-ocean environments will be degraded very slowly and persist for long periods of time.

CASE HISTORIES

The fate of petroleum hydrocarbons in the environment from various actual environmental oil contamination incidents has now been ex-

amined. It is extremely complex to study the weathering of a mixture such as petroleum in natural, variable environments. Patchiness of oil distribution and uncertainty about localized environmental variations make definitive scientific conclusions difficult to reach. Determining quantitatively the specific role of microorganisms in the fate of polluting oil is difficult, but changes in an environmentally contaminating oil can be viewed in light of the enzymatic degradative capacity of the indigenous hydrocarbon-degrading microbial populations. Environmental factors known to influence rates of microbial hydrocarbon degradation can be examined to estimate limitations of the biodegradative contribution to the removal of petroleum pollutants.

In February 1970, the tanker Arrow ran aground and spilled a large portion of its 108,000-barrel cargo into Chedabucto Bay, Nova Scotia. An estimated 300 km of shoreline was affected. Rashid (227) described the changes in the oil 3.5 years after the spillage. Degradation of oil depended largely on environmental factors, especially wave energy. Degradation was greatest in high-wave-energy environments and lowest in protected embayment areas. In the high-energy environments, there was a substantial loss of *n*-alkanes, which was believed to be due to microbial degradation. Presumably, oxygen and nutrients replenished by wave-driven mixing permitted more extensive degradation. Six years after the spill, it was impossible to estimate the amount of oil remaining in Chedabucto Bay from the spillage due to the patchy distribution of the oil, contributions of more recent spillages, and the absence of adequate control sites (170).

In January 1973, the Irish Stardust ran aground near Vancouver Island, B.C. Approximately 180 metric tons of fuel oil was spilled. Cretney et al. (84) examined the long-term fate of the heavy fuel oil from the spill that contaminated a British Columbia, Canada, coastal bay. They reported that biodegradation accounted for almost complete removal of *n*-alkanes during the first year after the spill. Pristane and phytane were biodegraded more slowly, but were almost completely gone after 4 years. The non-*n*-alkane components of the C_{28}-to-C_{30} range appeared to be the most resistant to degradation of all the components resolved by gas chromatography.

During March 1971, a pipeline rupture allowed JP-4 jet fuel and no. 2 fuel oil to enter the intertidal zone of a cove at Searsport, Maine. The spill was approximately 13 metric tons, but only a fraction of that amount probably entered the cove. Mayo et al. (194) examined the weathering characteristics of petroleum hydrocarbons deposited in fine-clay marine sediments of the cove. They found that petroleum residues isolated from the spill gave the appearance of weathering particularly slowly in the cold anoxic sediment. In 1976, they found that the average area contained roughly 20% less hydrocarbon than in 1971, when the spill occurred. At a number of sites, there appeared to be no decline in gross hydrocarbon concentrations and essentially no weathering of the aliphatic portions of the petroleum residues. The data indicated that although microbial degradation of the aliphatic linear chain systems had a measurable impact on the residues contained in upland sediments, this action was greatly suppressed in the residues absorbed on the anoxic cold clay silt of the cove. Microbial degradation apparently played a role during transport of the oil from the upland spill location to the marine sediment, but within the marine sediments rates of microbial degradation must have been near zero. It is likely that a lack of available oxygen in the contaminated sediments severely limited rates of biodegradation.

The tanker Metula grounded in the Straits of Magellan in August 1974. Approximately 46,000 metric tons of oil was lost, contaminating a cold marine environment. Colwell et al. (75) examined the biodegradation of petroleum from the Metula spill in the Straits of Magellan region. They found from biodegradation studies that oil degradation under in situ conditions proceeded relatively slowly, with marked persistence of Metula oil in the Straits of Magellan 2 years after the spill. They reported that the slow rates of oil degradation most probably were due to limitations imposed by relatively low concentrations of nitrogen and phosphorus available in seawater, as well as restricted accessibility to degradable compounds within aggregated oils or tar balls. Temperature did not seem to be a limiting factor for petroleum degradation in the cold marine environment. There was an indigenous cold-adapted microbial community capable of utilizing hydrocarbons. Microbial degradation was not effective in attacking buried oil or oil that had formed asphalt layers on beaches. Microbial action may have contributed significantly to the formation of polar material and contributed to the extensive removal of aliphatic hydrocarbons in favorable environments. It was concluded that the oil from the Metula spill would persist for a long period of time.

Two major spillages of no. 2 fuel oil into Buzzards Bay, Mass., have been studied. The Florida created the West Falmouth spill in 1969, and the Bouchard was the source of a second spill in 1974. Blumer et al. (41–43) examined the disappearance of oil from the West Falmouth spill. They found that the disappearance of petroleum hydrocarbons was slow and that bacte-

rial degradation contributed to the removal of *n*-paraffins. Teal et al. (262) examined the aromatic hydrocarbons contaminating the sediments of Buzzards Bay resulting from both spillages. Microbial degradation was believed to contribute to the disappearance of naphthalenes with zero to three alkyl substituents and phenanthrenes with zero to two substituents from surface sediments. The more substituted aromatics decreased relatively less and probably were more resistant to biodegradation.

Pierce et al. (220) examined the persistence and biodegradation of fuel oil on an estuarine beach which came from the spillage of 90,000 gal (ca. 342,000 liters) of no. 6 fuel oil into Narragansett Bay, R.I., in 1973. The concentrations of hydrocarbons in the midtide region declined simultaneously with an increase in populations of hydrocarbon-utilizing bacteria. During the winter months, hydrocarbon biodegradation was apparent at rates of less than 1 μg of hydrocarbon per g (dry weight) of sediment per day. McAuliffe et al. (196) examined the fate of 65,000 barrels of crude oil spilled in 1970 from a Chevron platform 11 miles (ca. 17.6 km) east of the Mississippi River delta. They found that only 1% of the oil entered the sediments; much of the oil dissipated. One week after the spill, there was evidence for biodegradation of the oil in the sediment as shown by an alteration in the ratio of *n*-paraffins to isoprenoid hydrocarbons. Within 1 year, most of the oil was gone and rapid biodegradation appeared to contribute to the removal of contaminating hydrocarbons.

Atlas et al. (21) studied petroleum biodegradation in various coastal Arctic ecosystems which had been experimentally contaminated with Prudhoe crude oil. Hydrocarbon biodegradation potentials were lower in ice than in water or sediment. Natural rates of degradation were slow, and maximal losses from experimental oil spills were less than 50% during the Arctic summer due to combined abiotic and biodegradative losses. Rates of biodegradation were found to be limited by temperature and concentration of available nitrogen and phosphorus. Residual oil had similar percentages of hydrocarbon classes as fresh oil; i.e., biodegradation of all oil component classes, including paraffinic and aromatic fractions, apparently proceeded at similar rates. In March 1977, there was a spill from the Potomac into the ice-laden waters of Melville Bay in the northeastern part of Baffin Bay, off western Greenland. About 107,000 gal (ca. 406,600 liters) of Bunker C fuel oil was lost. The fate of the oil was investigated by a team of scientists (129). Biodegradation of the oil at the low water temperatures was found to proceed very slowly if at all. There was no significant increase in numbers of hydrocarbon utilizers within a few weeks after the spill. During this period there was also almost no change in the C_{17}/pristane ratio in the oil, indicating that biodegradation was not occurring at a significant rate.

The spill of the supertanker Amoco Cadiz in March 1978 resulted in the largest oil spill to that date. In excess of 190,000 metric tons of oil was released into the marine environment during 2 weeks. A variety of intertidal sites off the Brittany coast was affected. Aminot (3) examined the fate of the oil in the water column before reaching the shoreline. He found a depletion of N, P, and O_2 in the water column beneath the oil, which apparently resulted from microbial degradation of petroleum hydrocarbons. The in situ deficits of N, P, and O_2 converted to a hydrocarbon biodegradation rate of 0.03 mg of oil degraded per liter per day in the water column under the oil. Aminot estimated that 9,000 metric tons of oil was biodegraded in the water column during the 2 weeks following the spill. The fate of the Amoco Cadiz oil within the intertidal zone was studied by several investigators (18, 19, 44, 55, 269, 288). Microbial degradation appears to have played a very important role in the weathering of oil stranded within the littoral zone. Atlas and Bronner (19) estimated a biodegradation rate of 0.5 μg of hydrocarbon per g (dry weight) of sediment per day within the affected interidal zone. The onset of extensive changes in the oil appears to have occurred more rapidly after the wreck than was anticipated, extensive biodegradation even preceding complete evaporation and dissolution of volatile aromatics (18, 55); there was a rapid change in the *n*-alkane/isoprenoid hydrocarbon ratio within days to weeks. The isoprenoid alkanes, C_{27} to C_{31} *n*-alkanes, hopanes, alkylated dibenzothiophenes, and alkylated phenanthrenes were the classes of hydrocarbons most resistant to biodegradation. Despite the rapid rates of biodegradation, the magnitude of the spill was such that the oil will persist within the littoral zone for a prolonged period. Oil that was buried, oil within anoxic sediments, and oil within embayments appears to be most persistent (18, 44, 280). Conditions which enhance aeration and resupply nutrients, such as high-energy wave action, favor biodegradation.

The magnitude of the Amoco Cadiz spill was surpassed by the spill from the IXTOC-I well blowout. In June 1979, oil began spilling into the Bay of Campeche, Gulf of Mexico. The oil flowed for 10 months before the well was capped. Some of the oil washed onto the coastal beaches of Texas, but for the most part the current carried the oil away from U.S. waters. The oil from the IXTOC-I well formed a mousse. Boehm and

Fiest (45) found little evidence for biological weathering of the hydrocarbons in the mousse. Atlas and co-workers (22, 23) found that biodegradation of mousse was greatly restricted, probably due to nutrient limitations and limited surface area for microbial attack. During a 6-month laboratory incubation under simulated natural conditions, 2 to 5% of the mousse (73) was converted to CO_2. Despite favorable temperatures and high populations of hydrocarbon utilizers in association with the mousse, changes in *n*-alkane/isoprenoid ratios took months rather than days to weeks. The contribution of biodegradation to weathering of oil from the IXTOC-I well was notably slower and of less magnitude than was found for the Amoco Cadiz. Pfaender and co-workers (52, 219) examined the degradation of hydrocarbons within the water column affected by the IXTOC-I oil. They found relatively rapid turnover times for hydrocarbons which had become dissolved in the water column. Rates of degradation ranged from 0.01 to 44 μg of aliphatic hydrocarbon respired per liter per h with turnover times of 30 to 266 h.

In contrast to the cited studies on large marine oil spills, there have been few studies on freshwater oil spills. Hydrocarbon contamination of freshwater ecosystems occurs frequently, but the spillages are generally of small magnitude. Unless a special resource such as a drinking water supply is contaminated, such "minor" spillages are often neglected. Jamison et al. (154, 155) did examine the degradation of gasoline in a contaminated groundwater supply. They used stimulated biodegradation to enhance removal of hydrocarbons from the contaminated water supply. Roubal et al. (240) followed the disappearance of hydrocarbons from the Ohio River after a major spillage of gasoline. They found that the hydrocarbons were rapidly removed. The microbial community was found to be capable of contributing to the disappearance of the contaminating hydrocarbons; the biodegradative potential was capable of responding within 1 to 2 days. Horowitz and Atlas (146) examined the fate of 55,000 gal (ca. 209,000 liters) of gasoline which had contaminated an Arctic lake that served as a drinking water supply. In situ measurement of gasoline degradation showed that, if untreated, sediment retained even "volatile" light hydrocarbons. Nutrient addition was found to enhance biodegradative losses.

Several studies have examined the fate of oil in soil ecosystems. Some of these studies involved experimental contamination of soil to examine the feasibility of using land farming for removal of oily wastes (100, 114, 130, 171, 182, 193, 229). Concern has been expressed about the leaching of oil applied to soil into groundwater supplies. There have been some reports on mobilization of oil into the soil column (271), but in most cases there has been little evidence for significant downward leaching of oil (100, 229). Kincannon (171) applied residual oil from a refinery tank, Bunker C fuel oil, and a waxy raffinate to soils and found a degradation rate of 8.3 $m^3/4 \times 10^3$ m^2 per month. Francke and Clark (114) reported a degradation rate of 11.9 $m^3/4 \times 10^3$ m^2 per month for used crankcase oil applied to soil. Raymond et al. (229) conducted extensive field tests to examine the optimal conditions for oil degradation in soil. They found that rates of degradation did not exceed 2.4 $m^3/4 \times 10^3$ m^2 per month.

Dibble and Bartha (102) examined the rehabilitation of a New Jersey wheat field which had been contaminated with approximately 1.9 million liters of kerosene over 1.5 hectares. A rehabilitation program consisting of liming, fertilizing, and frequent tilling was initiated, and the decrease of hydrocarbon contaminants was monitored for a 2-year period. During the 2 years of the study, the hydrocarbon content of the surface oil decreased to an insignificant level. Seasonal differences were found in the rate of hydrocarbon disappearance. Within 1 year after the spillage, the field returned to a near-normal productive state. Odu (211) reported evidence for microbial degradation of oil spilled on a sandy soil in Nigeria from an oil well blowout. Several Arctic terrestrial oil spills have been examined. Cook and Westlake (78) found evidence for extensive utilization of *n*-alkanes in oils applied in the Norman Wells area of the northwest territories and in the Swan Hill area of northern Alberta. They also found evidence for biodegradation of oil of the Nipisi spill in northern Alberta. The spill was on a sphagnum bog. Sexstone et al. (252), in contrast, found evidence for greatly restricted rates of biodegradation in northern soils. They found that hydrocarbons were still present in soils at Fish Creek, Alaska, 28 years after contamination by spillage of refined oil.

CONCLUSIONS

The rates of biodegradation of hydrocarbons from oil spills appear to be highly dependent on localized environmental conditions. It is apparent that the microbial degradation of oil pollutants is a complex process and that environmental factors have a great influence on the fate of spilled oil. The fate of many components in petroleum, the degradative pathways which are active in the environment, the importance of cooxidation in natural ecosystems, and the role of microorganisms in forming persistent environ-

mental contaminants from hydrocarbons such as the compounds found in tar balls are unknown and require future research. Although a number of rate-limiting factors have been elucidated, the interactive nature of microorganisms, oil, and environment still is not completely understood, and further examination of case histories is necessary to improve predictive understanding of the fate of oil pollutants in the environment and the role of microorganisms in biodegradative environmental decontamination. With an understanding of the microbial hydrocarbon degradation process in the environment, it should be possible to develop models for predicting the fate of hydrocarbon pollutants and to develop strategies for utilizing microbial hydrocarbon-degrading activities for the removal of hydrocarbons from contaminated ecosystems.

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